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4. Title of the invention — VACCINE COMPOSITION

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Vaccine Composition

The present invention relates to a composition which is particularly useful for delivering medicaments and particularly vaccines to mucosal surfaces, for example intranasal formulation. The invention further comprises methods of treating individuals using the composition and methods of preparing the composition.

A prime objective in the field of vaccination is the development of a non-parenteral immunisation regimen which facilitate induction of comparable levels of systemic immunity to that elicited by conventional sub-cutaneous and intra-muscular injections.

The nasopharyngeal passages and pulmonary regions of the respiratory tract represent potential targets for the systemic delivery of peptidergic drugs and vaccines. The relative ease with which therapeutic agents can be inhaled, or introduced into the nose, make these modes of immunisation attractive in terms of probable patient compliance. Furthermore, respiratory mucosae offer certain morphological, physiological and immunological advantages over other non-parenteral sites in terms of immunisation, particularly against pathogenic entitities which affect or utilise mucosal surfaces as portals of entry. This is because effective vaccination against these pathogens normally requires mucosae to the adequately protected with locally produced antibodies of the secretory IgA (sIgA) isotype. Whilst mucosal surfaces are usually poorly protected with IgA following parenteral administration of vaccines, it is now apparent that successful delivery of antigenic material to immunoresponsive elements in mucosa-associated lymphoid tissue (MALT) can result in vigorous stimulation of the mucosal arm of the immune system. By means of the common mucosal immune system (CMIS) it is feasible that several anotomically disparate mucosal surfaces could be protected through mucosal

administration of a vaccine at a single site. Mucosal vaccination offers the added advantage that some degree of systemic immunity can be induced in concert with local responses due to translocation of antigenic material from sub-epithelial compartments to systemic immunoresponsive tissues.

Despite the logistical and immunological factors which favour non-parenteral immunisation, simple mucosal application of antigenic proteins, for example in the gastrointestinal or respiratory tracts, is usually ineffectual in terms of vaccination. Enzymatic or chemical destruction, combined with poor absorption into sub-epithelial compartments dictate that mucosally administered vaccines usually require some form of adjuvant or delivery vehicle. One approach is to encapsulate antigenic material within microparticulate polymeric carriers, such as poly-DL-lactide (PLA) microspheres (Vaccine 1994, 12, 5-11). Such procedures serve to protect labile vaccines from lumenal degradation and enhance adsorption into mucosal and systemic compartments (J.H. Eldridge et al., Seminars in Hematology, (1993), 30, 16-25). There is good evidence that microencapsulation may also adjuvantise by converting soluble antigenic molecules into particulate species, thus promoting vaccine uptake into antigen presenting cells (APC)(Y. Tabata et al., Adv. Polym. Sci. (1990), 94, 107-141, L. Vidard et al., J. Immunol. (1996), 156, 2809-2818, N. Van Rooijen, Immunol. Today (1990) 11, 436-439) or microfold cells (M-cells) in lymphoid follicles (R.I. Walker et al., Vaccine, 12, 387, 1994, D.T. O'Hagan et al,, Vaccine, 1989, 7, 421-424, P.G. Jenkins et al., J. Drug Targetting, 1995, 3, 79-81). Nasal delivery of microsphere formulation of vaccine has also been reported (A.J. Almeida et al., J. Pharm & Pharmacology, 25, 198-203 1993, H.O. Alpar et al., J. Drug Targeting 2/2, 147-149, 1994, A.J. Almeida et al., J. Drug Targeting 3(b), 255-467 1996).

Although until recently comparatively under-investigated, the intra-nasal (i.n.) route is an attractive one for the mucosal delivery of vaccinal entities. The nasal epithelium is accessible and is less exclusive to high molecular weight molecules.

The thickness of the mucus blanket covering respiratory epithelium is relatively thin compared to that of other mucosae, for example the gut where it is in the region of 500 times thicker. Substantially reduced concentrations of proteolytic enzymes and extremes of pH exist in the respiratory tract compared with the gastrointestinal tract.

Furthermore, it is now delineated that nasal associated lymphoids tissues (NALT) have a lymphoepithelium which, like that in the intestinal mucosa, contain M-cells for selective antigen uptake (P. Brandenburg, Immunology of the Lung and Upper Respiratory Tract, (ed. Bienenstock J.) McGraw-Hill, New York, 1984, 28-95). Hence NALT plays an analogous role to other MALT, such as the gut associated lymphoid tissues (GALT), in terms of antigen surveillance and induction of mucosal and systemic immunological responses.

The applicants have found that a particular range of chemicals, when included in formulations, can enhance the effect of biologically active agents and in particular vaccines when administered to mucosal surfaces.

According to the present invention there is provided a pharmaceutical composition for application to a mucosal surface, said composition comprising

(i) a biologically active agent;
(ii) a chemical which enhances the effect of the biologically active agent wh n administered to a

mucosal surface, said chemical being soluble in water and being selected from one or more of:

A) a soluble derivative of a polycationic carbohydrate,

B) a clathrate,

C) a complexing agent,

D) a polyamino acid,

F) a vitamin or vitamin derivative,

G) cationic pluronics,

H) cetrimides; or

I) Methyl-glucamine

(iii) a pharmaceutically acceptable carrier or diluent, provided that when the chemical (ii) above is selected from B) or C), the biologically active agent is an agent which is capable of generating a protective immune response in an animal to which it is administered.

Administration to mucosal surfaces may be effected by oral application, by pulmonary application, for example by intra-tracheal administration, or particularly by intra-nasal application. In particular, the compositions of the invention are administered by the intra-nasal route.

Examples of suitable chemicals which enhance the mucosally produced effect of the biologically active agent are polymeric or other materials which may act as absorption enhancers and/or bioadhesive compounds and/or solubilisers. Examples of such compounds include

A) water-soluble derivatives of polycationic carbohydrates in particular, water-soluble chitin derivative such as a alkylated chitosan derivatives and salts thereof,

B) clathrates in particular cyclodextrins and their derivatives such as dimethyl β cyclodextrin,

C) complexing agents such as bile salts, in particular those which form complexes with fatty acids such as deoxycholic acid,

D) polyamino acids such poly-ornithine, for example of molecular weight from 5 to 150kDa;

E) vitamins or vitamin derivatives such as vitamin E TPGS (d-alpha tocopheryl polyethylene glycol 1000 succinate);

F) cationic pluronics, which are block copolymers or surfactants which are positively charged, in particular with NH_2^+ groups;

G) cetrimides which are quaternary ammonium compounds used as preservatives; or

H) methyl-glucamine.

Preferably the chemicals include at least one of the group selected from (A), (B), (C), (D) or (E). When the chemical comprises a vitamin or derivative of (E) above, it is suitably present in concentrations in excess of 0.2%w/v, preferably in excess of 2%w/v.

Examples of chemicals falling within category (A) above include trimethyl chitosan chloride, carboxymethyl chitosan, N-carboxymethyl chitosan, and polycethylene glycol chitosan. N-trimethyl chitosan chloride (TMC) has been referred (Kotze, A. F. et al. Pharm Res. (1997) 14:1197-1202) as a potential absorption enhancer of peptide therapies across mucosal membranes. In contrast to chitosan, TMC is water soluble in all gastrointestinal pH environments. Further, it retains the ability to temporarily open tight junctions. An important parameter appears to be the degree of trimethylation. Previously, we have demonstrated that the intranasal (IN) route is a highly effective, non-invasive alternative, to the parenteral administration of recombinant subunit vaccines (F1 and V derived from the causative organism of plague: *Yersinia pestis*) (Eyles, J. E. et al. Vaccine (1998) 16:698-707; J. Drug Targeting Nasal delivery of vaccines) 1996 Alpar & Almeida). Fraction 1 (F1) subunit (molecular mass 15.5 kDa) is derived from the capsule that surrounds the bacteria. In solution, because of its hydrophobic nature, F1 tends to aggregate into multimeric complexes of high (>200,000 kDa) molecular weight

(Voronisov, E. D. et al. Biomed. Sci. (1990) 1:391-396 and Miller, J. et al. FEMS Immun. Med. Micro. (1998) 21:213-231). The V antigen (molecular mass 37 kDa) is a protein secreted by the bacterium at 37°C (Leary, S. E. C. et al. Infect. Immun. (1995) 63:2854-2858). V is a virulence factor which may exert local anti-inflammatory effects through modulation of tissue cytokine levels (Nakajima, R. and R. R. Brubaker Infect. Immun. (1993) 61:23-31). Both F1 and V are protective, and there is a documented 'synergistic effect' in combination (Williamson, E. D. et al. Vaccine (1996) 14:1613-1619). Traditional killed whole cell vaccines for plague have an unsatisfactory incidence of transient local and systemic side effects, but more importantly, may fail to protect individuals from the pneumonic form of the disesase, which is transmissible *via* airborne droplets (Perry, R. D. and J. D. Fetherston. Clin. Microbiol. Rev. (1997) 10:35-66). Intramuscular injection (Williamson, E. D. et al. Vaccine (1997) 15:1079-1084) of combined F1 and V, or intranasal administration of microspheres co-encapsulating F1 and V (Eyles, J. E. et al. Vaccine (1998) 16:698-707), can protect experimental animals against a lethal inhalational challenge with *Y. pestis*.

Following these experiments it was seen that systemic immunity, in the form of high serum IgG titres to F1 and V, was critical for protection. In the current work, the applicants have nasally instilled F1 (5 μg) and V (1 μg) in the presence and absence of a variety of chemicals including three different TMC derivatives (with increasing degrees of quaternization: 20, 40 and 60%), and compared humoral immune response engendered by these treatments to those evoked by co-administration of chitosan itself.

Carriers or diluents used as (iii) above may vary depending upon the particular nature of the biologically active agent (i) and the further chemical (ii). They may comprise pharmaceutically acceptable solvents such as water in which the biologically

active agent (i) and the further chemical (ii) are dissolved. This type of formulation is particularly suitable when (i) is also water-soluble.

Compositions in the form of solutions of this type suitably contain from 0.1 to 30% w/v and preferably from 1 to 20% w/v of component (ii) above, depending upon the solubility of component (ii).

For many applications however, it has been found preferable that components (i) and (ii) are microencapsulated in a polymeric material and thus the carrier (iii) is a particulate carrier such as a microcapsule, nanocapsule or liposome.

Thus in a particular embodiment, the invention provides a pharmaceutical composition for administration to mucosal surfaces, which composition comprises particles comprising

(i) biologically active agent;

(ii) a material which enhances the biological effect of the composition, said chemical being soluble in water and being selected from one or more of:

A) a soluble derivative of a polycationic carbohydrate,

B) a clathrate,

C) a complexing agent,

D) a polyamino acid; or

F) a vitamin or vitamin derivative: and

(iii) a material capable of forming a particle.

Particularly suitable particles are liposomes and microspheres. Liposome forming chemicals for use as (iii) above are well known in the art and include lipids with a hydrophilic end region and a hydrophobic region and th opposite end of the molecule. Microspheres or microcapsules will generally be prepared using polymeric materials as is known in the art.

Suitably, the material which enhances the biological effect of the composition in this case is a polymeric material which is different to the polymeric material, where present, of item (iii) above.

The polymeric material (iii) above used in the compositions of the invention is may comprise one or more polymers, for example having molecular weights of from 2kDa or more. In particular, the polymeric material (iii) is a high molecular weight polymer, for example of molecular weight in excess of 94kDa, for example of 100kDa or more.

The use of high molecular weight polymers in the encapsulation of a tetanus vaccine for intramuscular administration has been described (Vaccine 1994, 12, 4, 299-306). A formulation of microencapsulated ricin toxoid vaccine which is applied intranasally has also been described (Vaccine 1994, 14, 11 1031). However, in that case, high molecular weight polymer microcapsules (94kDa) were less effective than those prepared from a copolymer of lower molecular weight (72kDa).

A particularly suitable polymeric material for use in the compositions of the invention comprises poly-(L-lactide) or PLA but other polymeric materials such as poly(lactic/glycolic acid) PGLA, polycyonacrylates, polyanhydrides or polycaprolactones as are known in the art may be employed.

Suitably the component (b) is present in the composition in an amount of from 0.1% to 10%w/w.

The compositions of the invention may optionally further comprise agents which stabilise emulsions such as polyvinylalcohol or methyl cellulose.

Other conventional reagents may be added. These include oth r known composition components such as colouring agents and

preservatives and in particular cetrimide. These are suitably present in amounts of from 0.1 to 0.7%w/v.

In a particular embodiment, the microspheres or liposomes used in the compositions may further comprise a coating of S-layer proteins, in particular, S-layer proteins derived from a bacteria against which the biologically active agent produces a protective immune response. It has been shown (Sleyr et al., Crystalline bacterial cell surface proteins. Biotechnology Intelligence Unit, 1996, R.G. Landes Company and Academic Press Inc.) that the stability of liposomes can be increased by such coatings. S-layer proteins are found on the surface of most bacteria and form a regular two dimensional array known as an S-layer. Isolated S-layer proteins are able to form entropy driven monomolecular arrays in suspension, and on the surface of structures such as liposomes.

Compositions of the invention are particularly suitable for intranasal application. They may comprise simple solutions of the components as described above, or microcapsules per se which are optionally preserved, for example by lyophilisation, or the microcapsules may themselves be combined with a pharmaceutically acceptable carrier or excipient. Examples of suitable carriers include solid or liquid carriers as is understood in the art.

Microcapsules used in the compositions of the invention will suitably be of an average size of from 0.1μm to 10μm in diameter.

These compositions may be used to deliver a range of biologically active agents including drugs and pharmaceutical chemicals as well as hormones such as insulin.

These compositions have been found to be particularly effective in the administration of biologically active agent which is capable of generating a protective immune response in an animal, particularly a mammal, to which it is administered. Examples of

such agents include antigenic polypeptides as well as nucleic acid sequences which may encode these polypeptides and which are known as "naked DNA" vaccines. Both the level and the longevity of the immune response is enhanced when these formulations are employed.

As used herein the expression "polypeptide" encompasses proteins or epitopic fragments thereof.

Suitable polypeptides are sub-unit vaccines, such as diptheria toxoid , tetanus toxoid and *Bacillus anthracis* protective antigen (PA).

In a preferred embodiment, the composition of the invention comprises a biologically active agent which is capable of generating a protective immune response against *Yersinia pestis*. The agent is suitably a sub-unit vaccine, for example as described in WO 96/28551. The vaccine described and claimed there comprises a combination of the V antigen of *Y. pestis* or an immunologically active fragment thereof or a variant of these, and the F1 antigen of *Y. pestis* or an immunologically active fragment thereof or a variant of these.

As used herein, the term "fragment" refers to a portion of the basic sequence which includes at least one antigenic determinant. These may be deletion mutants. One or more epitopic region of the sequence may be joined together.

The expression "variant" refers to sequences of nucleic acids which differ from the base sequence from which they are derived in that one or more amino acids within the sequence are substituted for other amino acids. Amino acid substitutions may be regarded as "conservative" where an amino acid is replaced with a different amino acid with broadly similar properties. Non-conservative substitutions are where amino acids are replaced with amino acids of a different type.

Broadly speaking, fewer non-conservative substitutions will be possible without altering the biological activity of the polypeptide. Suitably variants will be at least 60% homologous, preferably at least 75% homologous, and more preferably at least 90% homologous to the base sequence. Homology in this instance can be judged for example using the algorithm of Lipman-Pearson, with Ktuple:2, gap penalty:4, Gap Length Penalty:12, standard PAM scoring matrix (Lipman, D.J. and Pearson, W.R., Rapid and Sensitive Protein Similarity Searches, *Science*, 1985, vol. 227, 1435-1441).

Preferably, vaccine compositions will further comprise a further known adjuvant in order to enhance the immune response to the biologically active material administered. Suitable adjuvants include pharmaceutically acceptable adjuvants such as Freund's incomplete adjuvant, aluminium compounds and, preferably adjuvants which are known to up-regulate mucosal responses such as CTB, the non-toxic pentameric B subunit of cholera toxin (CT).

A particular aspect of the invention comprises a method of producing a pharmaceutical composition, which method comprises encapsulating a biologically active agent in a particle comprising a first material which is capable of forming a particle, in the presence of a chemical which enhances the effect of the biologically active agent when administered to a mucosal surface, said chemical being soluble in water and being selected from one or more of:

A) a soluble derivative of a polycationic carbohydrate,
B) a clathrate,
C) a complexing agent,
D) a polyamino acid; or
F) a vitamin or vitamin derivative.

Preferred examples of particle forming materials and effect enhancing chemicals are as set out above.

Methods of forming liposomes are well known in the art. They include dispersion of dehydrated phospholipid films into an aqueous medium, emulsion techniques and lyophilisation methods as are well known in the art.

The effect enhancing chemical may be incorporated within the microcapsule, or at the surface, of preferably is distributed throughout the microcapsule including the surface.

Microencapsulated compositions of the invention are suitably prepared using a double emulsion solvent evaporation method. Briefly, the biologically active agent, suitably in a lyophilised state, is suspended in an aqueous solution of the polymer such as polyvinyl alcohol (PVA) and the effect enhancer chemical. A solution of further polymer, in particular high molecular weight polymer in an organic solvent such as dichloromethane, is added with vigorous mixing. The resultant emulsion is then dropped into a secondary aqueous phase, also containing polymer (PVA or the like) and optionally also the enhancer material with vigorous stirring. After addition, the organic solvent is allowed to evaporate off and the resultant microspheres separated.

The compositions of the invention will suitably comprise an appropriate dosage unit of the active agent. This will vary depending upon the nature of the active agent being employed, the nature of the patient, the condition being treated and other clinical factors. In general however, the composition of the invention will comprise approximately 2 to 10 wt% of active ingredient.

In microcapsule formulations, the amount of high molecular weight first polymer in the composition will b of the order of 70 to 99wt% of the composition, and suitably from 90 to 99wt% of the polymer components will be the first polymer.

The amount of effect enhancing chemical present in the compositions will be sufficient to produce the required effect. This will vary depending upon the nature of the chemical but will generally be of the order of 0.1 to 10 wt % of the composition.

In use, a reasonable dosage for nasal administration would be of the order of 0.05g.

Preferred compositions of the inventions are vaccine compositions. Thus, in a further aspect, the invention provides a method of protecting a mammal against infection, which method comprises administration of a vaccine composition as described above to a mucosal surface, in particular an nasal surface, of a mammal.

The applicants have demonstrated that it is possible to protect experimental animals from inhalation challenge with various pathogens including diptheria, tetanus and *Y. pestis* through i.n. administration of a combined sub-unit vaccine. The adjuvantisation of these sub-units is advantageous in enhancing the immune response as is microencapsulation of the sub-units in accordance with the invention. The high molecular weight polymer utilised in the compositions of the invention appears to be particularly well suited to intra-nasal delivery.

The invention will now be particularly described by way of example with reference to the accompanying drawings in which:

Figures 1 and 2 illustrates the specific serum antibody responses following a single nasal application of 1μg V and 5μg F1 antigens of *Yersinia pestis* in compositions according to the invention:

Figures 3-5 illustrates the immune response to nasally delivered tetanus toxoid (TT)using compositions according to the invention

where BS is glycodeoxycholic acid, CYC is dimethyl β cyclodextrin, and VET is Vitamin E TPGS and PO is polyornithine;

Figures 6-7 illustrate the immune response to nasally delivered Diptheria toxoid (DT) using compositions according to the inventionS is glycodeoxycholic acid, CYC is dimethyl β cyclodextrin, and VET is Vitamin E TPGS and TT represents tetanus toxoid.

Example 1

Use of absorption enhancers to enhance immunological response to intranasally administered subunit vaccines

Five groups of five (n=5) BALB/c mice were intranasally immunised with admixed F1 (5 μg) and V (1 μg). The five treatment groups received the subunits in conjunction with either: 1) Phosphate buffered saline (pH 7.4); 2) 0.2% w/v chitosan HCL; 3) 0.2% w/v TMC 60; (4) 0.2% w/v TMC 40; (5) 0.2% w/v TMC 20. A further group of animals acted as a control.

Mice were lightly anaesthetised with an inhaled gaseous mixture of 3% (v/v) halothane (RMB Animal Health Ltd., UK) in oxygen ($300cm^3\ min^{-1}$) and nitrous oxide ($100cm^3\ min^{-1}$) for i.n. dosing procedures. Each mouse received a 15 μl volume of liquid administered with a micropipette. Tail vein blood samples were taken on day 14, and serum was analysed for the presence of anti-V and anti-F1 IgG antibodies using an indirect ELISA protocol (Eyles, J. E. et al. Vaccine (1998) 16:698-707).

The results (Fig. 1) indicate that mucosal co-administration of TMC60 or TMC40 augments the humoral response to F1 and V above and beyond that generated by i.n. instillation of F1 and V in phosphate buffered saline or chitosan HCL. TMC20 failed to improve titre to V, although the effect on immunity to F1 was comparable with that of the more substituted chitosan derivatives (TMC40 & 60).

Example 2

The effect of additional enhancing chemicals.

The procedure of Example 1 was followed, this time using the following compositions:

Treatment Groups

1 Antigens free in 138kDa polyornithine (0.2-1w/v conc.)
2 Microspheres including 138kDa polyornithine (0.2-1w/v conc.)
3 Free in 30-70KDa polyornithine (0.2%w/v)
4 Free in 5-15kDa polyornithine (0.2%w/v)
5 Free in β-cyclodextrins (2.5%w/v)
6 Microspheres in β -cyclodextrins (2.5%w/v)
7 Free in deoxycholic acid (0.25%w/v)
8 Free in Vitamin E TPGS (2.5%w/w)
9 Free in Vitamin E TPGS (0.2% w/v)
10 Free in chitosan HCL (0.2%w/v)
11 Free in phosphate buffered saline (PBS)
12 Microspheres in PBS

The results are shown in Figure 2. This clearly shows that other compounds, in particular, poly-L-ornithine either free or in microspheres, β-cyclodextrins, deoxycholic acid and Vitamin E TPGS (the latter being present in amounts of 2.5% w/v) produced enhanced results.

This study has identified that absorption enhancers, with potential applications for increasing the bioavailability of non-parenterally administered peptidergic drugs, can also act to improve humoral immunity to mucosally applied subunit vaccines.

Example 3

Immune responses to nasally delivered tetanus toxoid (TT)

Further tests were carried out using the methodology of Example 1 but replacing the *Yersinia pestis* antigens with tetanus toxoid. Mice were dosed on day 1 with 5 LF toxoid and on day

49 with 2.5 LF toxoid. The toxoids where in solution in combination with a variety of enhancing chemicals in various concentrations. The results are shown in Figures 3-5.

With these enhancers, titres for primary responses were improved approximately 100 times and secondary responses between 1000 to 20000 times compared to free antigen.

Example 4

Immune responses to nasally delivered diptheria toxoid (DT)

Example 3 was repeated on selected members of the enhancers using diptheria toxoid in place of tetanus toxoid. The results are shown in Figures 6 and 7. Again, similar levels of enhancement are noted.

Claims

1. A pharmaceutical composition for application to a mucosal surface, said composition comprising

(i) a biologically active agent;

(ii) a chemical which enhances the effect of the biologically active agent when administered to a mucosal surface, said chemical being soluble in water and being selected from one or more of:

A) a soluble derivative of a polycationic carbohydrate,

B) a clathrate,

C) a complexing agent,

D) a polyamino acid,

E) a vitamin or vitamin derivative,

F) cationic pluronics,

G) Cetrimides, or

H) Methyl-glucamine

(iii) a pharmaceutically acceptable carrier or diluent,

provided that when the chemical (ii) above is selected from B) or C), the biologically active agent is an agent which is capable of generating a protective immune response in an animal to which it is administered.

2. A composition according to claim 1 wherein which is adapted for intra-nasal administration.

3. A composition according to claim 1 or claim 2 wherein the said effect enhancing chemical can act as an absorption enhancer or bioadhesive compound or a solubiliser.

4. A composition according to any one of the preceding claims wherein the said chemical is selected from one or more of;

A) a water-soluble chitin derivative such as a alkylated chitosan derivatives and salts thereof,

B) cyclodextrins and their derivatives such as dimethyl β cyclodextrin,

C) complexing agents which form complexes with fatty acids such as deoxycholic acid,

D) poly-ornithine, for example of molecular weight from 5 to 150kDa; or

E) vitamins or vitamin derivatives such as vitamin E TPGS (d-alpha tocophenyl polyethylene glycol 1000 succinate).

5. A composition according to any one of the preceding claims wherein the carrier comprises a particle.

6. A composition according to claim 5 wherein the particle is a microsphere or liposome.

7. A compositon according to claim 6 which comprises a microsphere.

8. A composition according to claim 7 wherein the microsphere is prepared using a high molecular weight polymer.

9. A composition according to claim 8 wherein the polymer has a molecular weight of 100kDa or more.

10. A composition according to any one of claims 7 to 9 wherein the microsphere comprises poly-(L-lactide).

11. A composition according to any one of the preceding claims wherein the ratio of the chemical (ii) to the carrier is from 99:1 to 9:1 w/w.

12. A composition according to any one of the preceding claims wherein the biologically active is capable of generating a protective immune response in a mammal to which it is administered.

13. A composition according to claim 12 which further comprises an adjuvant.

14. A composition according to claim 13 wherein the adjuvant is the non-toxic B-subunit of cholera toxin.

15. A method of producing a prophylactic or therapeutic vaccine, which method comprises encapsulating a polypeptide which is capable of producing a protective immune response in a first polymeric material which has a high molecular weight, in the presence of a second polymeric material which enhances the biological effect of the composition.

16. A method of protecting a mammal against infection, which method comprises administration of a composition according to any one of claims 8 to 10 to a mucosal surface of a mammal.

16. A method according to claim 15 wherein the mucosal surface comprises an intranasal surface.

Abstract

A pharmaceutical composition for administration to mucosal surfaces, which composition comprises

(i) biologically active agent;

(ii) a chemical which enhances the effect of the biologically active agent when administered to a mucosal surface, said chemical being soluble in water and being selected from one or more of:

A) a soluble derivative of a polycationic carbohydrate,

B) a clathrate,

C) a complexing agent,

D) a polyamino acid,

E) a vitamin or vitamin derivative,

F) cationic pluronics,

G) cetrimides (quaternary ammonium compounds preservatives) or

H) methyl-glucamine

(iii) a pharmaceutically acceptable carrier or diluent,

provided that when the chemical (ii) above is selected from B) or C), the biologically active agent is an agent which is capable of generating a protective immune response in an animal to which it is administered.

The composition, which may be in the form of a solution or particles such as microspheres or lipsomes, is particularly useful for the intra-nasal administration of vaccines.

Figure 1

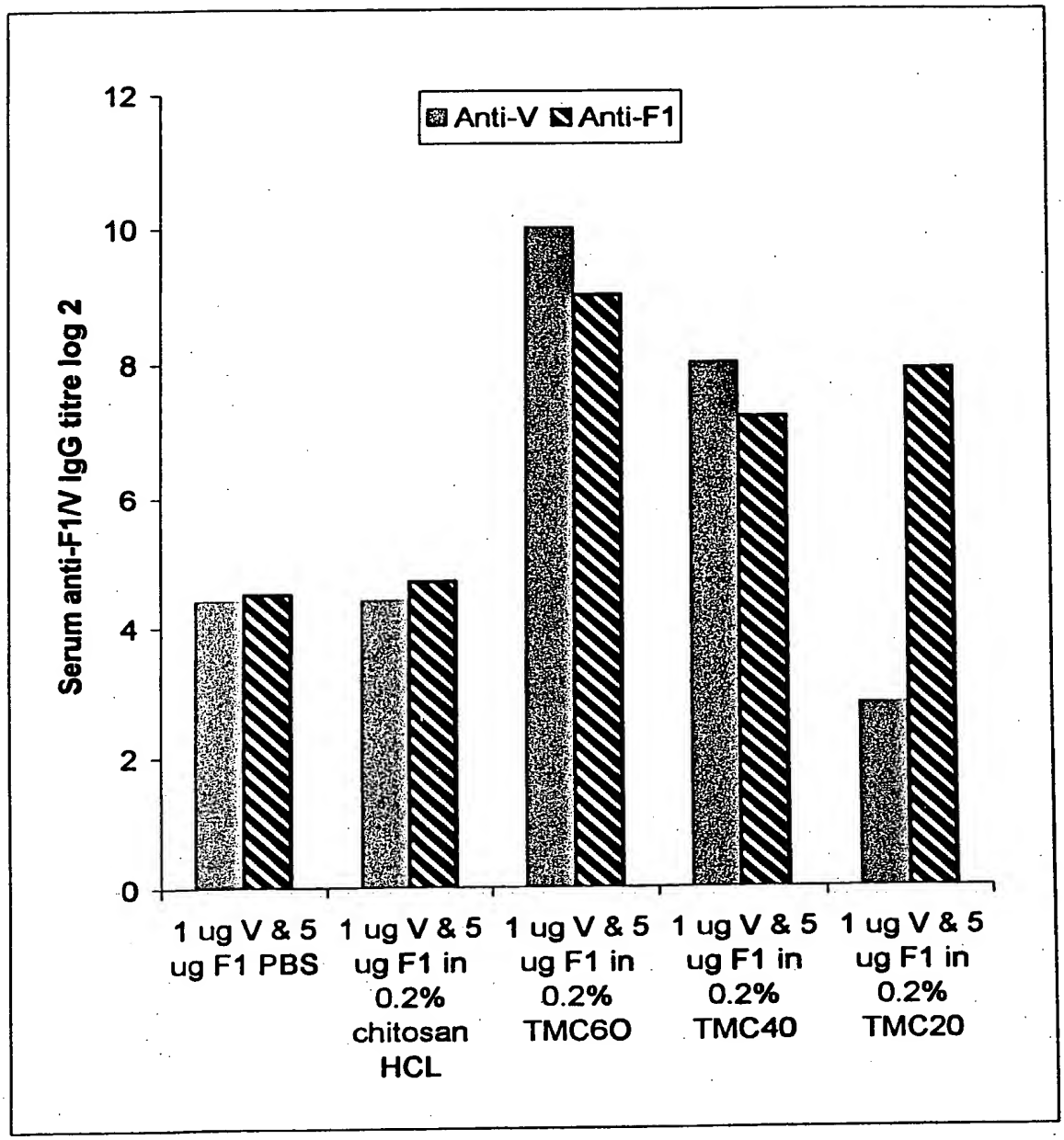

Anti-V
Anti-F1
Serum anti-F1/V IgG titre log 2
12
10
8
6
4
2
0
1 ug V & 5 ug F1 PBS
1 ug V & 5 ug F1 in 0.2% chitosan HCL
1 ug V & 5 ug F1 in 0.2% TMC60
1 ug V & 5 ug F1 in 0.2% TMC40
1 ug V & 5 ug F1 in 0.2% TMC20

Figure 2

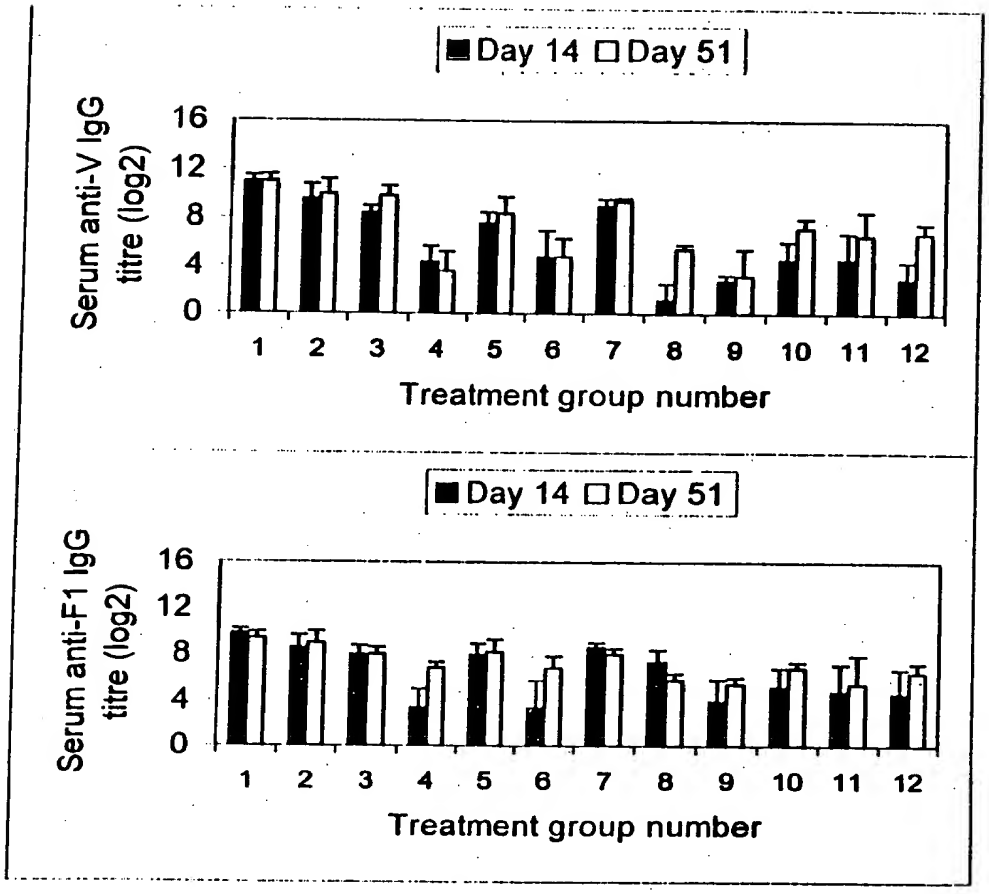
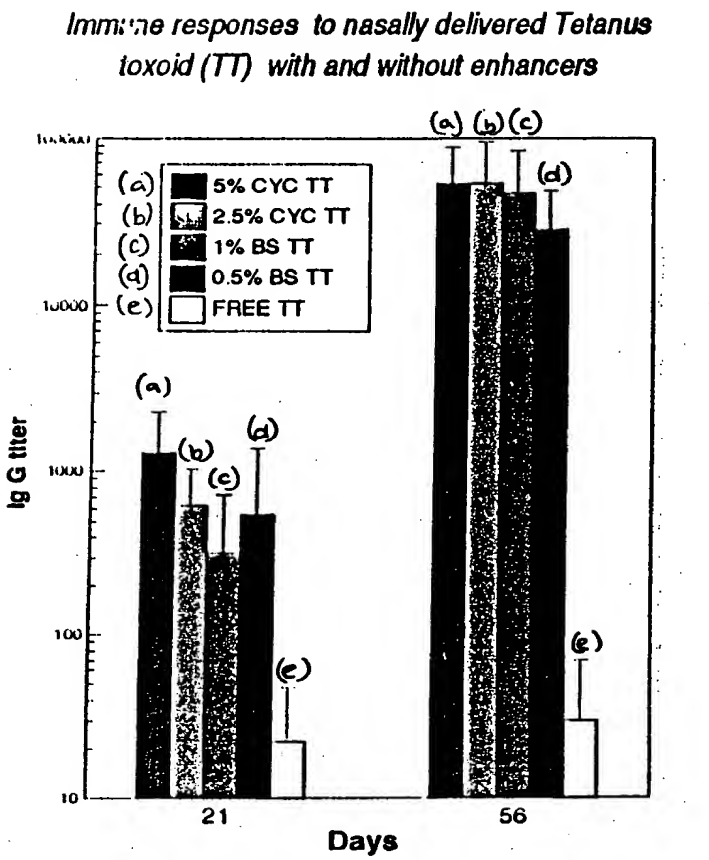

Day 14 Day 51
Serum anti-V IgG titre (log2)
16
12
8
4
0
1 2 3 4 5 6 7 8 9 10 11 12
Treatment group number
Day 14 Day 51
Serum anti-F1 IgG titre (log2)
16
12
8
4
0
1 2 3 4 5 6 7 8 9 10 11 12
Treatment group number

Figure 3

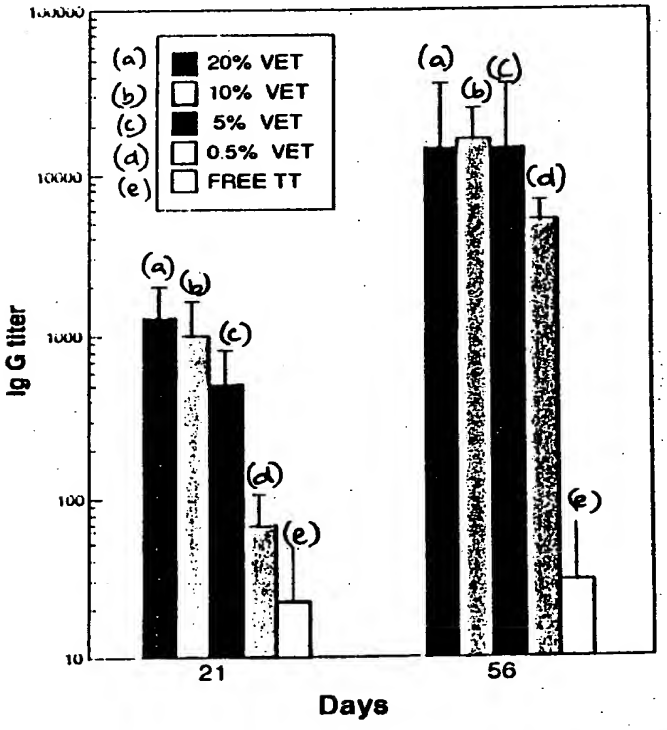


Mice dosed on day 1 with 5 LF toxoid and on day 49 with 2.5 LF toxoid

BS = Glyco deoxy cholic acid
CYC = Dimethyl (cyclodextrin

Figure 4

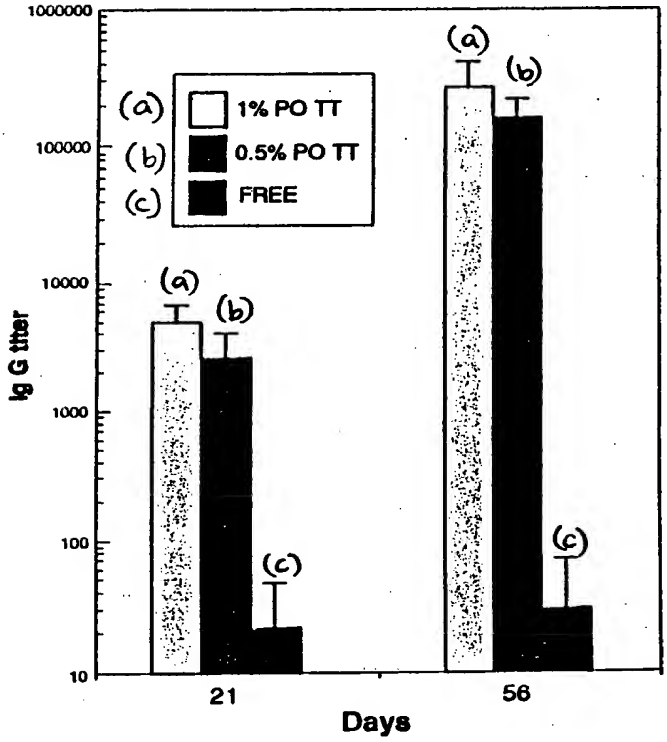
Immune responses to nasally delivered Tetanus toxoid (TT) with and without VitaminE TPGS (VET)



Mice dosed on day 1 with 5 LF toxoid and on day 49 with 2.5 LF toxoid

Figure 5

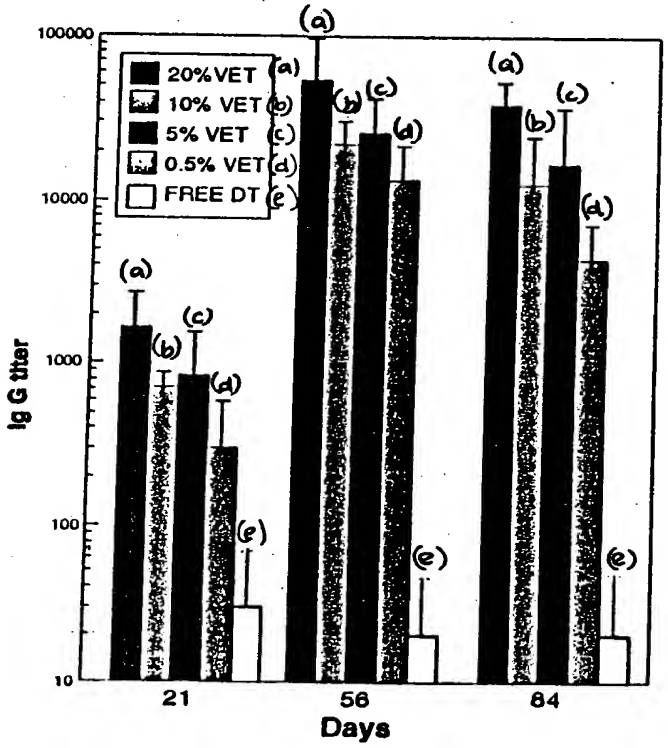
Immune responses to nasally delivered Tetanus toxoid (TT) with and without poly-ornithine (PO)



Mice dosed on day 1 with 5 LF toxoid and on day 49 with 2.5 LF toxoid

Figure 6

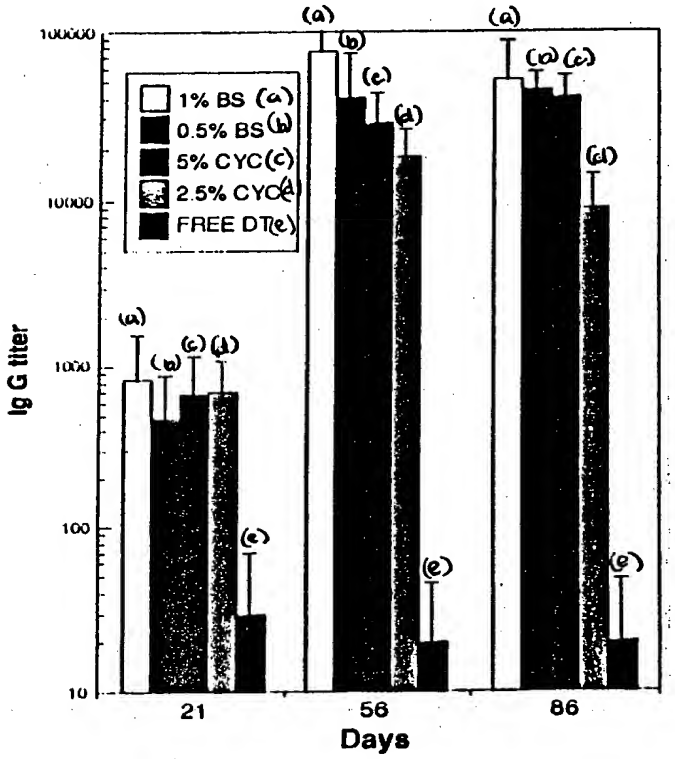
Immune responses to nasally delivered Diphtheria toxoid (DT) with and without VitaminE TPGS (VET)



Mice dosed on day 1 with 5 LF toxoid and on day 49 with 2.5 LF toxoid

Figure 7

Immune responses to nasally delivered Diphtheria toxoid (DT) with and without enhancers



Mice dosed on day 1 with 5 LF toxoid and on day 49 with 2.5 LF toxoid

BS = Glyco deoxy cholic acid
CYC = Dimethyl (cyclodextrin

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2/3/100